Brief Notes on the Structure and Development of Spirochæta anodontæ Keysselitz.

By

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With Plate 15.

Much uncertainty still prevails as to the structure and biological relationships of the spirochætes. Earlier observers —not altogether influenced perhaps by a desire to prove their affinities with the hæmoflagellates—equipped them with an undulating membrane and a complicated nuclear apparatus, the existence of which is very problematical; and much controversy has existed as to their mode of division, longitudinal or transverse. The very claim of the organisms found in Mollusca, as well as that of the smaller spirochætes, to be called by this name, depending as it must on their relationship to Sp. plicatilis Ehrenberg, the type-species, is not very firmly established, since the structure of the latter—a system of large waves, each broken by smaller undulations superposed on them, with a central axial rod and a series of dots, regarded as nuclei—is markedly different from that of the later additions to the genus. It is desirable, therefore, to record any observations which may tend to throw light on the morphology and life-history of these organisms.

My own observations have been carried out on Sp. anodontæ, a species easily obtained, and one which may be regarded as fairly typical of the spirochætes which are parasitic in Mollusca.

With regard to the so-called undulating membrane of

Sp. anodontæ I am inclined to agree with Schellack, who holds that the appearance which has been thus interpreted is an artifact, due to splitting of periplast, since I found that the better my preparations were fixed, the fewer were the examples of this condition. It was found almost invariably in films dried in the air (fig. 1), but very exceptionally in those fixed in osmic vapour. It seems possible that the periplast, if it is so to be called, adheres to the slide in the position in which death of the spirochæte occurs, and that subsequently the protoplasm of the organism shrinks and thus straightens its outline a little, leaving a line of periplast following a different line from that finally adopted. At other times the periplast seems to shrink most and to form a band uniting the cells of the spirochæte (fig. 2).

I have seen a considerable number of specimens which exhibit a dark line running along one side (figs. 3, 4). This probably corresponds with the "crest" described by Gross. Judging from the illustrations of spirochætes in section given by him and previously by Fantham, it would seem that the organism possesses a sheath which is loose enough to form a fold along one side in certain conditions. The sheath or periplast stains more darkly than the body-substance of the spirochæte, and hence the double layer appears as a dark line. Splitting of the sheath into fibrillæ seems sometimes to occur, as in fig. 6.

On the other hand, I find it difficult to agree with Schellack that two separate species of spirochæte are present together in Anodonta, one having blunt (figs. 1, 3, 4) and the other sharp extremities (figs. 7, 8, 9). The amount of difference seen is scarcely sufficient to suggest a specific differentiation. Further, the great variation in the length and thickness of the individuals in each class thus formed is such as to negative the possibility of distinguishing species by length, as he further suggests. It is noteworthy that Schellack distinguishes two species of spirochæte in more than one kind of mollusc, and that Gross also describes two, very similarly differentiated, in Pecten jacobæus. This rather strongly suggests that

the same spirochæte may present different shapes and sizes. I have never seen specimens of Sp. anodontæ with transverse lines near the middle of the length of the organism, pointing to transverse division by means of a septum here formed, as is depicted by Schellack.

As to the actual method of division, I had the opportunity of observing an instance of what appeared to be longitudinal fission. This was in a preparation containing living spirochætes in a drop of fluid from the mantle-cavity of the mussel. My attention was attracted to an individual which remained fixed at one point, near to the edge of the preparation, and wriggled feebly, instead of dashing about like the others. I then noticed that this spirochæte seemed to possess two "tails," if this expression is allowable (fig. 11*a*); and on watching it I noticed that a line, dark at one moment, refringent at another, as the creature moved, was gradually extending along the body of the spirochæte from the point of bifurcation towards the opposite extremity. Fission proceeded along this line, but not quite regularly as in tearing a strip of calico, since at one moment there was visible a sort of loop in the "anterior" part of the organism—using this term for the portion which remained single longest—before the process of division had extended quite to this point from "behind" (figs. 11*b*, *c*). When the longitudinal fissure had extended nearly to the "anterior" end, the organism suddenly jerked itself away out of the field of the microscope-and I was unable to see the final separation of the daughter, individuals; but as I was unable, on searching through the preparation, to find the dividing spirochæte again, I could only conclude that the separation took place almost immediately, the two resulting spirochætes being then indistinguishable from the other individuals present.

A possible source of error in this observation lies in the slenderness of the organisms and in the difficulty of accurately observing them during movement. It is scarcely possible to exclude the explanation that an organism may become folded upon itself as a preliminary to undergoing transverse fission

(figs. 12, 13). This alternative possibility was strongly brought to my mind by a preparation in which many living spirochætes appeared to have loops at one extremity, while they revolved rapidly on a longitudinal axis. As their movements slackened, these organisms appeared to have a shape which might be compared with that of a hair-pin having its "legs" twisted together. On fixing and staining this preparation, specimens were found both of the condition just described (fig. 14), and also of what seemed to be a succeeding stage, in which division had taken place at the bend of the loop (fig. 15). I could find no instance of a spirochæte simply looped at one extremity, as might have been expected to occur if longitudinal division took place, starting at a point a little distant from the extremity of the spirochæte. I was at first tempted to regard the pairs of closely apposed organisms here seen as being instances of conjugation, but the explanation just given seems more probable in view of the looped forms seen alive in the preparation. Division by "incurvation" is stated by Gross to occur in the spirochæte present in Pecten jacobæus (called by him Cristispira pectinis), and he believes it to be the characteristic of all spirochætes. Nuttall, Fantham and Porter state that in the small spirochætes both transverse and longitudinal division occurs. If this be so, it would be interesting to ascertain whether any difference in environment is responsible for their adoption of each method respectively, or whether the two processes occur at different points in the life-cycle.

With regard to the internal structure of Sp. anodontæ, I have always found that specimens taken from the crystalline style of the mussel stain homogeneously like Bacteria (fig. 7). Those, however, which are present in the stomach of the mollusc, especially after the style has dissolved, show beading of the substance of the organism, as is depicted by Keysselitz in his original memoir (figs. 16, 17, 18). Since the style is apparently the most favourable medium for the life of the spirochæte, I am inclined to look upon the beaded forms as either involutionary or developmental. The fol-

lowing observation supports the view that the change is, in some cases at least, one of development.

A piece of crystalline style was dissolved in tap-water and sealed up with vaseline for forty-eight hours under a cover-slip on a slide. At the end of this period the cover was removed, and the preparation was fixed in osmic vapour and stained with thionine. The spirochætes, which had originally been very numerous, were found to have almost entirely disappeared, while their place was taken by elongated, variously curved bodies, closely corresponding with them in length and thickness, containing irregular masses of staining substance (figs. 19 and 20). Some definite spirochætes present showed beading of protoplasm and separation into segments (segmentation, plasmolysis?), and it was possible to trace a fairly definite series of gradations between such spirochætes, as seen in this and other preparations, and strings of bead-like or coccoid bodies, which seemed to constitute the final stage of development (figs. 16, 18, 21, 22). It seems then that Sp. anodontæ goes through a stage in development in which it breaks up into coccoid bodies, just as, according to Leishman, Sp. duttoni breaks up into similar bodies inside the body of the tick. If this be so we have, on the one hand interesting evidence of the generic unity of the small and large spirochætes, and on the other, of the affinity of the whole group with the Bacteria rather than with the Protozoa.

The portion of style used for the above observation was taken from a mussel which had been kept at a temperature just above freezing-point. The spirochætes seemed specially numerous and lively at this temperature, and dividing forms were numerous. I also found some organisms in the water in which the mussels were kept. Most of these were stiff and motionless, but one or two individuals were actively moving when first seen. The addition of water to a piece of the style seems to have the effect of rendering a large number of the organisms motionless and rigid (fig. 23), and it is tempting to speculate as to whether the difference between

what has been called a Treponema, in which the curls are more or less rigid, and a Spirochæte, in which there is more flexibility, may not be dependent to some extent on the medium in which the organism is examined. Accompanying the spirochætes in the water were a considerable number of spirillar organisms, exactly resembling them in microscopical structure (figs. 23, 24 *a*, *b*). It seems possible that the segmenting spirochætes (fig. 19) may divide first into short spirilla in some instances before undergoing development into the coccoid stage. At a low temperature the style of the mussel is more slowly dissolved than at the temperature of the room. I have found that, as a rule, this body entirely disappears in about a week or ten days after the mussel is taken up from its native place. One mussel, which was kept for three weeks in an aquarium, where presumably food was plentiful, formed a new style, so far as could be judged, since all the other mussels of the same batch had lost theirs long before. This re-formed style was quite free of spirochætes.

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EXPLANATION OF PLATE 15,

Illustrating Dr. W. Cecil Bosanquet's paper on "Brief Notes on the Structure and Development of Spirochæta anodontæ Keysselitz."

[All the figures, except Figs. 1 and 11, are from specimens fixed in osmic vapour and stained with thionin, and all are drawn at a uniform magnification of 2000.]

Fig. 1.—Spirochæta anodontæ, showing so-called undulating membrane. From a film dried in the air and stained with iron-hæmatoxylin.

Fig. 2.—Sp. anodontæ—long thin specimen from crystalline style.

Fig. 3.—Same. Long thick specimen.

Fig. 4.—Same. Short thick specimen.

Fig. 5.—Same. Short thin specimen.

Fig. 6.—Same, showing splitting of (?) sheath.

Fig. 7.—Same. Slender specimen showing uniformity of structure and absence of "undulating membrane," like a bacterium.

Fig. 8.—Same. Short specimen with pointed ends.

Fig. 9.—Same. Longer specimen with pointed ends and somewhat stiff curls (not well shown in the figure).

Fig. 10.—Same. Short thin specimen with apparent undulating membrane.

Fig. 11.—*a*, *b*, *c*, stages in apparent longitudinal division of Sp. anodontæ (diagrammatic).

Figs. 12, 13.—Stages in "incurvation" (?) of Sp. anodontæ.

Figs. 14, 15.—Complete incurvation (?) and subsequent transverse division.

Figs. 16, 17, 18.—Sp. anodontæ, from the alimentary canal of the mussel, showing plasmolysis (?).

Figs. 19, 20, 21, 22.—Apparent development of spirochætes into coccoid bodies. From a preparation of crystalline style dissolved in water and kept for forty-eight hours.

Fig. 23.—Spirochæte from water in which mussels had been kept, for comparison of structure with figs. 24 *a*, *b*, showing spirilla from same water.